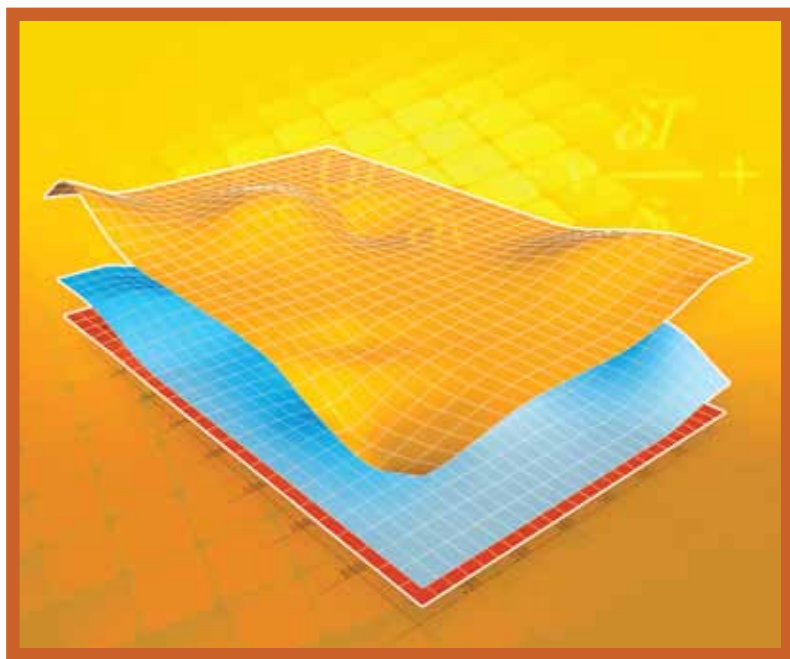


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Preparation of Dipalmitin by Fractionation Method

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ABSTRACT

Dipalmitin was prepared by glycerolysis of palmitic acid in the presence of isooctane and Lipozyme RM IM and was further purified by neutralization followed by fractionation. Dipalmitin containing samples was named as 'diacylglycerol chemical method' (DGCHM). The dipalmitin content in DGCHM was 71.83%, however, after fractionation, this percentage was raised to 89.80%. The DGCHM and RBDPO (refined bleached and deodorized palm oil) added with DGCHM were analysed for their crystallization behavior. The complete melting temperature for DGCHM was 70.7°C. The combined DSC and wide-angle XRD of the dipalmitin revealed the polymorphic transformation from α to sub α to α . The yield for DGCHM was 71% (w/w). The purification of dipalmitin by fractionation method was considerably practical due to the higher yield, easier preparation, shorter preparation time and smaller amounts of starting materials required. The addition of 1% DGCHM to RBDPO has a negligible effect on its crystallization and heating properties.

Keywords: dipalmitin, differential scanning calorimetry (DSC), X-ray diffraction (XRD), purification, refined bleached and deodorized palm oil (RBDPO)

INTRODUCTION

Diacylglycerols (DAG) are esters of the trihydric alcohol glycerol in which two of the hydroxyl groups are esterified with fatty acids [1]. In food industries, DAG have been widely used as food emulsifier (owing to its excellent emulsifying properties) [2]. In margarine, it may be used to improve the texture of the margarine [3]. DAG oil is also manufactured as a functional cooking oil in Japan and has been marketed in countries such as Japan and United States [4,5]. DAG, especially 1,3-DAG have a number of beneficial effects on lipid metabolism and the intake can lead to reduce in body weight and visceral fat accumulation in rats and human [6]. In non food application, DAG have been used in cosmetics and pharmaceutical products as drug carriers [2].

DAG have been produced either by chemical, enzymatic or a combination of both methods. DAG preparations by chemical method involved reacting carboxylic acids with glycidyl esters or glycidal ethers in the presence of an ammonium salt [7]. Haftendorn and Ulbrich-Hofmann [8] synthesized 1,3 dilauryolglycerol and dimyristoylglycerols by reacting glycerol and vinyl esters of carboxylic acid in the presence of *Mucor miehei*. Several other lipases have also been screened for optimum production of DAG [4, 9, 10]. Yang et al., [6] reported the production of DAG from butterfat by glycerolysis and short path distillation. Acyl donors such as ethyl caprate, capric acid and tricaprins for dicaprin production [11], ethyl stearate for distearin production [12], fatty acids from palm oil deodorizer distillate [10] hydrogenated beef tallow [13] and triolein for dioleyolglycerol synthesis [14] have been reported. In addition, parameters affecting DAG formation including time, temperature, molecular sieves, water content, enzyme load and types of solvent have also been studied [10, 15, 16].

1,3-dipalmitin has also been prepared previously to study its polymorphic behavior where the preparation involved reacting acid chlorides from pure fatty acids with pure glycerol followed by crystallization in ether or hexane [17]. However, no further detail studies of the dipalmitin produced were reported. In another study, hardened palm oil was used and subjected to glycerolysis process [18]. However, the resulting mixture have to be stored for 24 hr to achieve DAG content at 85 % concentration with the remaining 15 % composed of monoacylglycerol (MAG) and triacylglycerol

(TAG). Other dipalmitin preparations involved long preparation time of at least 24 hr [9, 19]. This study outlines a method for the synthesis of large amount of dipalmitin of reasonable concentration for bulk applications in fractionation studies. Preparation of dipalmitin by fractionation has never been reported before. Dipalmitin was prepared by glycerolysis of palmitic acid in the presence of a lipase followed by neutralization and fractionation. Palmitic acid was used as an acyl donor and some of the characteristics of the dipalmitin produced were studied. In addition, the effects of the prepared dipalmitin at the concentrations of 0, 1, 3 and 6 % on the crystallization behaviors of palm oil were also reported.

MATERIALS AND METHODS

Materials

Palmitic acid was obtained from Southern Acids Sdn Bhd (Selangor, Malaysia), lipase Lipozyme RM 1M from *Rhizopus miehei* was kindly provided by Novozyme (Copenhagen, Denmark) through Science Technics Sdn Bhd (Selangor, Malaysia) and molecular sieves (3Å, 1/16 inch pellet) and standards comprising 1,3-dipalmitin, 1,2- dipalmitoyl-sn-glycerol, 1,2-dipalmitoyl-rac-glycerol, 1-monopalmitoyl-rac-glycerol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade while solvents were of high performance liquid chromatography (HPLC) grade.

Preparation of Dipalmitin

Dipalmitin was prepared by glycerolysis in the presence of solvent (isooctane) and Lipozyme RM 1M lipase. Sixty four grams of palmitic acid was placed into a beaker and 100 ml isooctane was added. The beaker and its content were then placed in a thermally controlled water bath (Haake, Germany) preset at 65 °C and left to completely melt the palmitic acid. 11.5 g glycerol anhydrous (2:1 molar ratio of palmitic acid to glycerol), 4 % Lipozyme RM 1M lipase (based on the total weight of palmitic acid and glycerol) and 10 % molecular sieves (based on the total weight of palmitic acid and glycerol) were added. To ensure complete mixing, the mixture was continuously stirred using a stirring motor (Model IKA RW11, Staufen, Germany). In the preliminary study, preparation of dipalmitin was

performed using the similar conditions as mentioned above except that the reaction times were varied at 2.5, 3, 3.5 and 4 hr. From the preliminary study, 3.5 hr was found to produce the highest amount of dipalmitin. Thus, this reaction time was used for further preparation of dipalmitin. The reaction was terminated by vacuum filtering through a Whatman #1 filter paper. The vacuum process was continued until the collected filtrate in the conical flask was dried to evaporate as much solvent as possible. The filtrate was further air dried and analysed for its acylglycerols composition by HPLC. This collected filtrate was considered as the “DGCHM before neutralization” and was further purified as follows:-

Purification by Fractionation Method

“DGCHM before neutralization” was subjected to neutralization with sodium hydroxide [20] to remove the unreacted free fatty acids and monopalmitin. This was followed by fractionation at 50°C for 1 hr. The dipalmitin prepared by this method was named as “DGCHM”.

Analysis of the Prepared Dipalmitin and RBDPO Added with DGCHM

DGCHM was analysed for its melting and crystallization behavior by differential scanning calorimetry (DSC) while its polymorphic behavior was analysed using the combined DSC and X-ray diffraction.

DGCHM was also added into RBDPO at 0, 1, 3 and 6 % and the melting and crystallization behavior, solid fat content (SFC), iodine value (IV), triacylglycerols (TAG) composition and fatty acid compositions of the RBDPO were studied. Polymorphic behavior of RBDPO and RBDPO added with 3, 6, 9, 12 and 15 % DGCHM were also studied using the combined DSC and X-ray diffraction.

High Performance Liquid Chromatography (HPLC)

TAG and DAG compositions were determined according to AOCS [21] (AOCS Method Ce 5c-93) by using HPLC equipped with auto sampler and a refractive index detector (Agilent 1100 Series, USA). Samples melted at 70 °C were prepared at a concentration of 0.05 g per ml of chloroform

and filtered through a 0.45 µm cellulose membrane filter. Five µl sample was injected into the LiChrospher® 100 RP-18e; 5 µm (250 mm × 4 mm) column. The oven temperature was set at 35°C while the flow rate was 1 ml / min, the mobile phase used was an acetone:acetonitrile mixture (75:25, v/v) run under isocratic condition. RBDPO (AOCS Lab Proficiency Program Palm Oil, Series Sample #1) was used as a reference for the retention time and quantification of the peaks in the reference and sample. The total running time was 40 min.

For the determination of acylglycerol compositions (monopalmitin, palmitic acid, dipalmitin and tripalmitin) in the prepared DGCHM samples, similar conditions were applied. 0.02 g of the sample to be analysed was weighed and dissolved in 1ml chloroform. Synthetic reference acylglycerols such as 1,3-dipalmitin, 1, 2- dipalmitoyl-sn-glycerol, 1,2-dipalmitoyl-rac-glycerol, 1-monopalmitoyl-rac-glycerol, tripalmitin and palmitic acid were used to identify the retention time for each corresponding peak. The relative percentage of each acylglycerol was calculated according to AOCS [21] as follows:-

$$\% \text{ Acylglycerol} = \frac{\text{Area of individual peak}}{\text{Sum of all peak area}} \times 100$$

Melting and Crystallization Behavior

DSC analysis was performed using the Perkin Elmer DSC-7 (Connecticut, USA) connected to Thermal Analysis Controller TAC-7/ DX Perkin –Elmer. About 5 to 8 mg of melted samples were weighed into aluminum pans which were then sealed using a sample pan crimper. Samples were initially held for 10 min at 80 °C to erase any past crystal memories. The temperature was then reduced to –50 °C at a rate of 5 °C/min followed by holding at -50 °C for 10 min to obtain the crystallization curves. After holding, the temperature was increased to 80 °C at a heating rate of 5°C/ min to obtain the melting curves. In the past, a DSC rate of 5°C/min was found to be the optimal rate in terms of length of time for analysis and the rate of thermal event in palm oil.

Polymorphic Form, SFC, Fatty Acids Composition and Iodine Value

The combined polymorphic behavior and thermal properties of dipalmitin (DGCHM) were determined using the combined DSC-XRD (Rigaku TTRAXIII XRD, Japan). The DSC measurement was performed at a rate of 5° C/min for both the cooling and heating runs in the temperature range of -40 to 80 °C. Rotating anode voltage and current were set at 50 kV and 300 mA, respectively. The temperature of the DSC was controlled by using liquid N₂. The XRD calibration was carried out using sodium behenate.

SFC, fatty acids composition and iodine value were determined according to PORIM Test Methods [20]. SFC determination was performed using the pulsed nuclear magnetic resonance (pNMR) spectrometry (Bruker Minispec P20:20 Mhz, Karlsruhe, Germany). Fatty acids composition was determined through direct methylation of the sample with sodium methoxide and methanol. The standard mixture of fatty acid methyl esters (RM-6, Supelco-Cat. No 07631-1 amp 10.0 mg, USA) was prepared by dilution with n-hexane prior to injection into a gas chromatography (GC). 0.1 ul sample was injected into the GC (Hewlett Packard 5890 Series II, USA) fitted with a 60 m × 0.25 mm × 0.25 µm Supelco SP-2340 capillary column. The injector and detector temperatures were set at 240 °C while the column oven was operated between 180°C-190°C. Helium gas carrier flow rate was 20 ml/min. Individual peaks of fatty acid methyl esters were determined with an FID detector and the running time was 53 min. Determination of iodine value was conducted using cyclohexane method according to PORIM Test Method Cd-25 [20]. 0.4 g RBDPO was weighed and dissolved in 20 ml cyclohexane. 25ml WIJS solution was added and the reaction was carried out in the dark for 1hr. Subsequently, potassium iodide solution was added to stop the reaction. The remaining iodine was titrated using 0.1N sodium thiosulphate.

RESULTS AND DISCUSSION

Dipalmitin Preparation by Fractionation

Table 1 shows the acylglycerols composition in DGCHM obtained before and after neutralization and fractionation. Monopalmitin and palmitic acid content was reported as sum of total monopalmitin and palmitic acid as reported by Lo et al. [10]. This was due to poor resolution of these acylglycerols where distinct separation of the respective elution regions could not be obtained. Also, there was still some amount of tripalmitin remaining in the reaction products probably due to difficult separation of DAG from TAG under normal circumstances [22]. In this study, probably the thermal properties of dipalmitin and tripalmitin are quite close to each other, thus separation of these two components was difficult to achieve. Tripalmitin crystallized at 44.9 °C [23]. Thus, the fractionation temperature applied (50 °C) in this study caused the crystallization not only of dipalmitin but also tripalmitin and as a result, some amounts of tripalmitin were also found in DGCHM.

As shown in Table 1, dipalmitin was found to be the main component of acylglycerol in DGCHM before and after neutralization. Similar observations where glycerolysis did not completely result in 100% composition of DAG has also been reported previously where glycerolysis of butterfat produced a composition of 7 % MAG, 86 % DAG and 7 % TAG [6]. Kristensen et al. [4] obtained 60 – 65 % DAG using lipase PS-D, lipase AK and lipase F-AP15. Commercial DAG oil produced by esterification of fatty acid with either glycerol or MAG in the presence of lipase or through the chemical glycerolysis of oil and fats in the presence of a chemical catalyst contains 80 % DAG and 20 % other components including TAG and MAG [5]. Monopalmitin (MP) and palmitic acid (PA) were observed to be removed by neutralization with sodium hydroxide followed by fractionation resulting in the increased of dipalmitin (DP) content from 71.83 % (before) to 89.8 % (after) neutralization and fractionation. The yield of DGCHM obtained was 71 % (w/w). Yield refers to weight percent of DGCHM collected (calculated as the weight of DGCHM collected after fractionation over the weight of palmitic acid used). The preparation of DP by the fractionation method was considered to be a practical method to produce DP for used in the fractionation studies. This was due to the higher yield with reasonably

high amount of DP in the DGCHM, easier preparation, shorter time and small amounts of the starting materials (DGCHM before neutralization) required.

Table 1: Acylglycerols (monopalmitin + palmitic acid, dipalmitin and tripalmitin) composition (%) in DGCHM produced before and after neutralization and fractionation at 50°C and 1 hr reaction time

Acylglycerols	Before neutralization and fractionation	After neutralization and fractionation
Monopalmitin + Palmitic acid	26.89 ±0.25	Not detected
Dipalmitin	71.83 ±0.76	89.80 ±1.01
Tripalmitin	1.57 ±0.05	10.20 ±0.09

Values represent means of three replicates ± standard deviation

DGCHM: diacylglycerol chemical method

Crystallization and Melting Behavior

The crystallization and melting curves of DGCHM are shown in Fig 1. Two exothermic peaks were observed in the crystallization curves of DGCHM with peak maxima at 59.9 °C and 43.0 °C. These peaks could be associated with the major components present in these compounds i.e. dipalmitin and tripalmitin and their associated crystallization behavior. DGCHM started to crystallize (onset of crystallization) at 61.5 °C. The melting curves of DGCHM also showed the presence of two endothermic peaks at 69.6 °C and 48.2 °C. DGCHM completely melted at 70.7 °C.

Complete melting temperatures described above referred to the endset of the melting curves. The onset of crystallization and complete melting temperatures of DGCHM as well as the peak temperatures obtained in this study matched closely with report by Swe *et al.* [24] . Their crystals of palm olein which were mainly composed of 1,3-PP had exothermic and endothermic peaks at 58.5 °C and 67.9 °C, respectively. Ours were 59.9 °C and 69.6 °C. While their onset of crystallization and complete melting temperatures were 61.4°C and 70.4 °C, respectively, and ours were 61.5 °C and 70.7 °C.

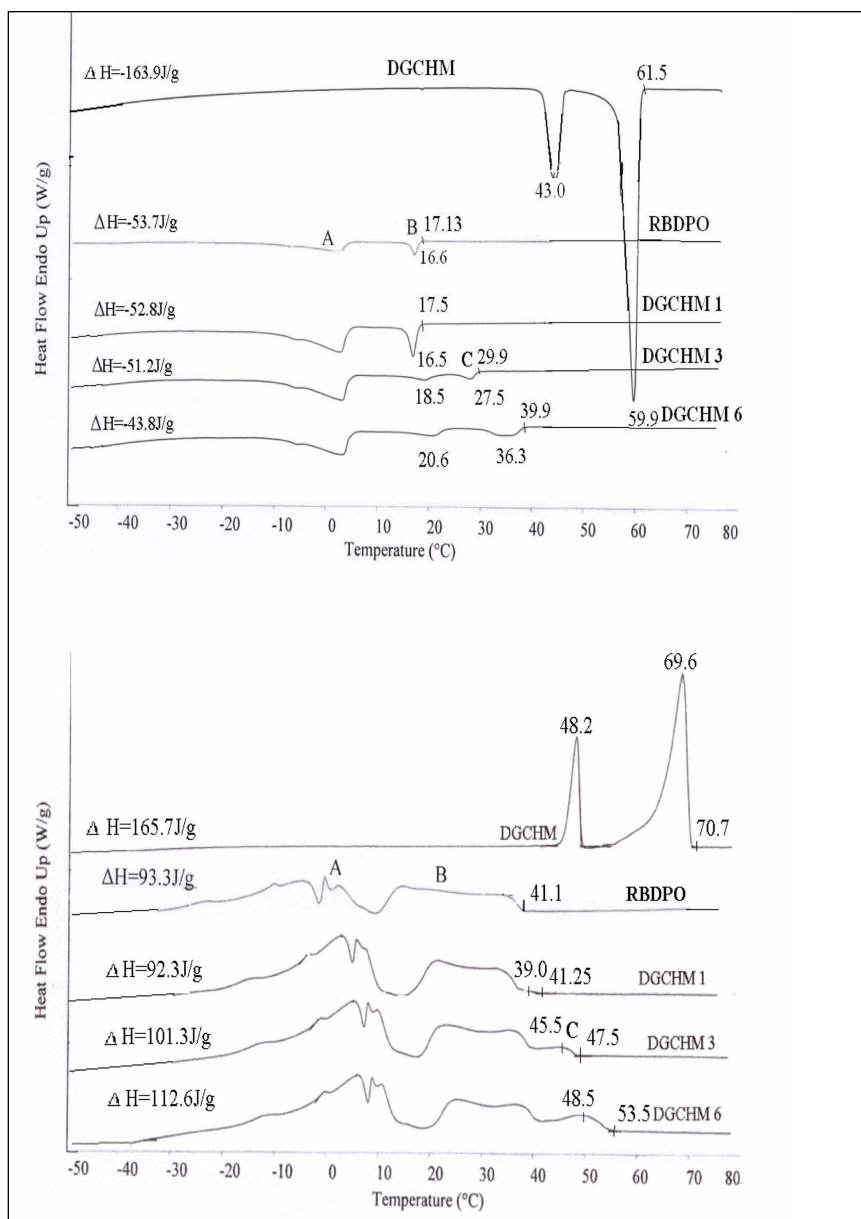


Figure 1: DSC Crystallization (top) and Melting (bottom) Curves of DGCHM and RBDPO added with DGCHM at 1, 3 and 6 %

DSC Crystallization and Melting Behavior of RBDPO and RBDPO added with DGCHM

The Effect of DAG Addition on RBDPO: The crystallization and melting curves of RBDPO added with 0, 1, 3 and 6 % DGCHM are shown in Fig 1. RBDPO crystallization curves showed the presence of two exothermic peaks i.e low-T peak (A) (olein) and high-T peak (B) (stearin). The addition of 1 % DGCHM has no effect on the crystallization of RBDPO where the onset of crystallization temperature was close to that of RBDPO. However, at 3 % addition, stearin peak was shifted to 18.5 °C and a new high-T peak (C) was observed at 27.5 °C with the onset of crystallization at 29.9°C. At 6 % DGCHM addition, stearin peak was further shifted to 20.6 °C and peak (C) also shifted to 36.3 °C with onset crystallization temperature at 39.9 °C. The increase in DGCHM addition from 3 to 6 % resulted in the increased in the onset temperature for crystallization by 10 °C. Peak (C) was observed to be broader as the concentration of DGCHM added is increased suggesting the presence of broader range of TAG compositions. The dipalmitin in DGCHM may probably act as a seeding agent that crystallized earlier and once it crystallized, it promoted the crystallization of RBDPO TAG especially those with lower melting points and thus enhancing the crystallization of RBDPO especially at higher percentages of DGCHM addition. The trends of the DSC crystallization curves were consistent with the decreased in IV (Table 2) suggesting that the addition of DAG formed harder RBDPO. No effect on the olein peaks was observed at all percentages of DGCHM added.

Table 2: Fatty Acid Compositions and Iodine Values of RBDPO added with 1, 3 and 6 % DGCHM

RBDPO	DGCHM	RBDPO + 1 % DGCHM	RBDPO + 3 % DGCHM	RBDPO + 6 % DGCHM
Saturated fatty acids (%)				
49.7±0.8	100±0.0	53.1±1.6	53.4±0.5	55.0±1.2
Unsaturated fatty acids (%)				
49.1±1.2	ND*	46.5±0.8	46.1±0.8	44.6±1.7
Iodine value				
53.1±0.1		53.9 ^A ±0.6	52.4 ^{AB} ±1.1	51.1 ^B ±0.8

Values represent means of three replicates ± standard deviation

Means within a row with different letters are significantly different (p< 0.05);

*ND = Not detected

The melting curves of RBDPO showed that RBDPO completely melted at 41.1 °C (Figure 1). Two endothermic peaks were present i.e low-T peak (A) (olein) and high-T peak (B) (stearin). At 1 % DGCHM, a new small high-T peak (C) appeared having peak at 39 °C and complete melting temperature shifted to a higher temperature (41.25 °C). With the addition of 3 % DGCHM, peak (C) became more prominent and complete melting temperature further shifted to 47.5°C. At 6 % DGCHM addition, peak (C) was observed to be broader and complete melting temperature further shifted to 53.5 °C. Thus RBDPO with the addition of 1, 3 and 6 % DGCHM completely melted at 41.25 °C, 47.5 °C and 53.5 °C, respectively. Peak (C) was most probably due to the excess of the high melting DGCHM crystallizing out of the solution first in the crystallization run and melting the last in the heating run due to its high melting point (70.7 °C). Oleins were not much affected by the DGCHM addition.

Polymorphic Behavior of DGCHM by Combined DSC and X-ray Diffraction

Figure 2 shows the combined DSC (heating and crystallization) curves and XRD of the prepared dipalmitin. The XRD curves showed that DGCHM transformed from α to sub α to α upon cooling and heating. Lutton [25] introduced the term sub α with short spacings at 4.14, 3.92, 3.75 and 3.56 Å for 1-monopalmitin and 1- monostearin. The transformation from α to sub α was reversible and occurred at low temperatures [25]. Sub α refers to β' form usually melt below an α form and it can be transformed either to α or to β form [25]. DGCHM started to crystallize in α form when cooled from 59.6 °C to 12.9 °C. The peak was sharp with short spacing at 4.13 Å and long spacing at 28.98 Å. There was a transient period where the peak started to separate at 12.9 °C. Further cooling to -26.4 °C separated the peaks into two and transformation into sub α (short spacings 4.14 Å and 3.7 Å and long spacings 49.4 Å, 24.7 Å, 16.3 Å, 12.3 Å and 8.15 Å) occurred. Subsequent heating at 22.9°C caused reversible transformation from sub α to α characterized by short spacing at 4.11 Å and long spacings at 49.46 Å, 24.91 Å, 16.22 Å, 12.27 Å and 8.21 Å. According to Baur *et al.* [26], the rate of polymorphic transformation depends on the purity of samples. Monoacylglycerols promote transformation while triacylglycerols exert a retarding effect [26]. Thus probably due to the presence of traces of tripalmitin in DGCHM (Figure 1) that possibly has the retarding effect,

the polymorphic transformation occurred was from α to sub α instead of from α to β' forms.

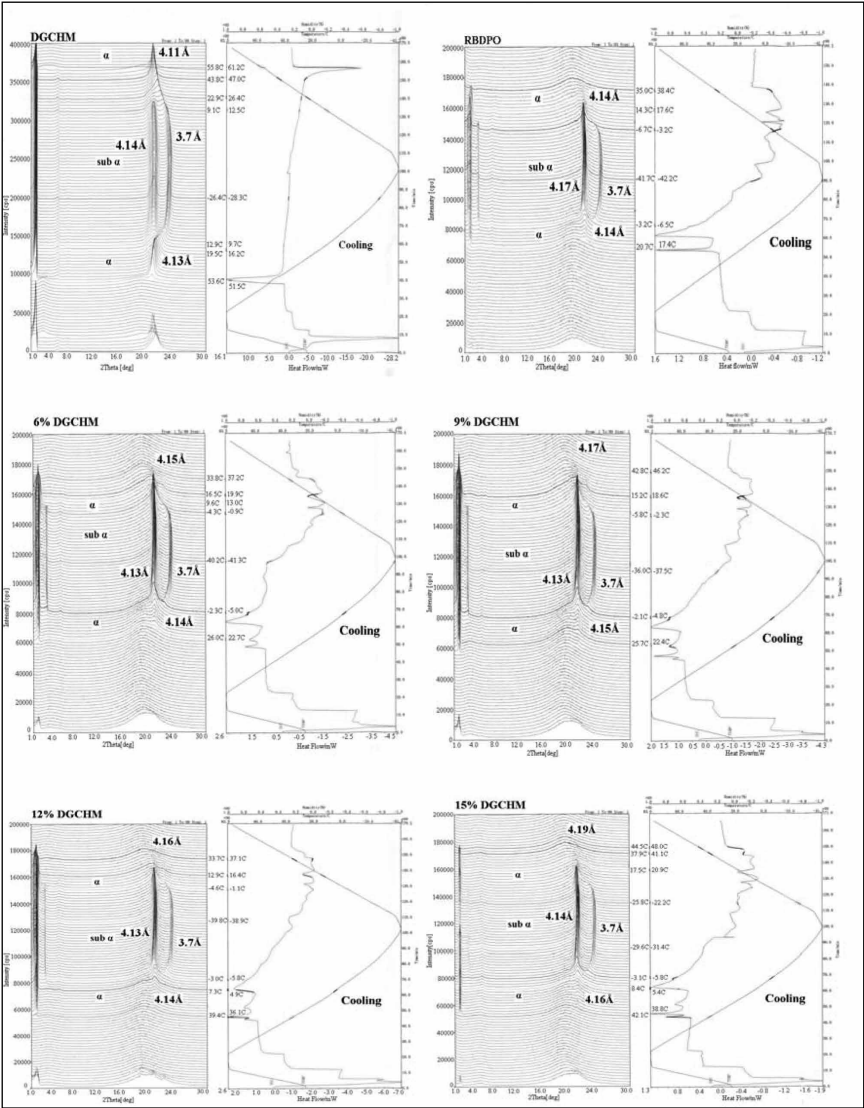


Figure 2: Combined DSC and X-ray Diffraction of DGCHM, RBDPO and RBDPO Added with 3, 6, 9, 12 and 15 % DGCHM

Polymorphic Behavior of RBDPO and RBDPO added with DGCHM by Combined DSC and X-ray Diffraction

Figure 2.4 show the DSC (heating and cooling) curves and XRD result of RBDPO and RBDPO with the addition of 3, 6, 9, 12 and 15% DGCHM. Upon cooling from 20.7 to -3.2 °C, RBDPO exhibits α form (4.14 Å). It was then transformed into sub α (short spacings at 4.17 Å and 3.7 Å; long spacings at 46.9 Å, 27.7 Å and 15.6 Å) when further cooled to -41.7 °C. At heating from -6.7 to 14.3 °C, the sub α -form reversibly transformed into the α -form (short spacing at 4.14 Å; long spacings at 46.6 Å and 15.5 Å). Sub α -form is made by cooling a fat that is already in the α -form to even lower temperatures [27]. The α -form is recovered when the temperature is raised again [27].

According to D'Souza et al. [28] short spacings at 3.7 and 4.19 Å is the characteristic of the β' 2 or sub α structure. Palm oil shows three polymorphs ie β' 2, β' 1 and α [29]. Since that the transformation of α to β' is slow, a slow cooling rate is required to observe this result. Fast cooling rate may result in the sub α -form as observed in this study with a cooling rate of 5 °C / min for the XRD-DSC runs. A cooling rate of 1 °C / min is recommended to be tried for future work.

RBDPO added with the DGCHM also showed similar polymorphic transformation from α to sub α to α . RBDPO added with 3, 6 and 9% DGCHM showed the α -form when cooled between 24.3 to 26 °C, however, when DGCHM concentration was increased to 12 and 15 %, the α -form appeared at 39.4 and 42.1 °C. This suggested that the higher the percentage of DGCHM addition, the higher is the melting point of α . Similar polymorphic transformation from α to sub α suggested that RBDPO and RBDPO added with DGCHM had similar crystal behaviors during crystallization. No different in polymorphic transformation between RBDPO and different percentages of DGCHM addition suggested that the differences in DSC crystallization and melting curves were not due to the polymorphic transition but due to the differences in chemical compositions (PPP, DGCHM content and saturated fatty acid compositions; Tables 2, 4 and 5) of the crystals formed. For margarine products, the desired polymorphic form is the β' -form giving smooth texture whereas β (large crystal) giving grainy structures are more suitable for chocolate products.

XRD patterns (Table 3) showed that RBDPO added with DGCHM contained mixtures of β' and β crystals. The addition of DGCHM induced the formation of β crystals. This was observed by the formation of β crystals which increased with increased in the percentage of DGCHM added up to 26°C. However, at 28°C, when the percentage of DGCHM was increased to 12 and 15%, the $\beta \geq \beta'$. The high melting β crystal is relatively more stable than the β' form and hence remains in the melt [30]. DGCHM showed the β polymorphic form. The β -form crystals have also been reported in the palm olein crystals containing 1,3-dipalmitin [24, 30]. Shannon et al. [31] obtained short spacings at 4.68 Å VS, 4.55 Å S, 3.91 Å S and 3.78 Å S for 1,3- dipalmitin and α -form (4.15 Å) for 1,2 –dipalmitin crystallized from the melt. Baur et al. [26] found two different β forms for dipalmitin recrystallized from hexane. These β form dipalmitins were characterized by strong short spacing (4.6 Å). Based on the strong short spacing (4.6 Å), they named the dipalmitin as β_a characterized by short spacings (4.6 Å VS, 3.9 Å M and 3.7 Å S) and long spacings (44 Å VS, 22.2 Å M, 14.9 Å S, 8.9 Å W, 7.5 Å W+ and 5.6 Å W+). β_b obtained from transformation of β_a was characterized by short spacings (4.6 Å VS and 3.75 Å S+) and long spacings (47.0 Å VS, 23.8 Å M, 15.9 Å S, 9.48 Å VW, 7.86 Å M and 5.97 Å W).

The Effect of DGCHM Addition on RBDPO: Chemical Properties

The TAG composition in RBDPO containing DGCHM is shown in Table 4 and 5. DGCHM addition did not show significant effect on the percentages of individual TAG as well as the monounsaturated (SSU), diunsaturated (SUU), triunsaturated (UUU) and trisaturated (SSS) TAG. The main TAG in palm oil are tripalmitoylglycerol (PPP, 5 %), 1,3-dipalmitoyl-2-oleoylglycerol (POP, 22 %) and rac-1-palmitoyl-2,3-dioleoylglycerol (POO, 22 %) [32].

Table 3: Polymorphic forms of RBDPO and RBDPO added with 3 to 15 % DGCHM and Determined at 18 to 28 °C

Temp. (°C)	Polymorphic forms						
	RBDPO	DGCHM	RBDPO + 3% DGCHM	RBDPO + 6% DGCHM	RBDPO + 9% DGCHM	RBDPO + 12% DGCHM	RBDPO + 15% DGCHM
18	β'	β	$\beta + \beta'(\beta' > \beta)$	$\beta + \beta'(\beta = \beta')$	$\beta + \beta'(\beta = \beta')$	$\beta + \beta'(\beta > \beta')$	$\beta + \beta'(\beta > \beta')$
20	β'	β	$\beta + \beta'(\beta' > \beta)$	$\beta + \beta'(\beta = \beta')$	$\beta + \beta'(\beta > \beta')$	$\beta + \beta'(\beta > \beta')$	$\beta + \beta'(\beta > \beta')$
22	β'	β	$\beta + \beta'(\beta' = \beta)$	$\beta + \beta'(\beta = \beta')$	$\beta + \beta'(\beta > \beta')$	$\beta + \beta'(\beta > \beta')$	$\beta + \beta'(\beta > \beta')$
26	β'	β	$\beta + \beta'(\beta = \beta')$	$\beta + \beta'(\beta = \beta')$	$\beta + \beta'(\beta > \beta')$	$\beta + \beta'(\beta > \beta')$	$\beta + \beta'(\beta > \beta')$
28	β'	β	$\beta + \beta'(\beta = \beta')$	$\beta + \beta'(\beta = \beta')$	$\beta + \beta'(\beta > \beta')$	$\beta + \beta'(\beta \geq \beta')$	$\beta + \beta'(\beta \geq \beta')$

DGCHM: diacylglycerol chemical method

RBDPO: refined bleached and deodorized palm oil

Table 4: Triacylglycerol (TAG) Composition (%) in RBDPO added with 1, 3 or 6 % DGCHM

	PLL	PPP	OLO	PLO	PLP	OOO	POO	POP	SOO	POS	PPS	*others
RBDPO	2.77	5.83	1.99	9.90	9.06	4.76	21.90	27.21	2.56	4.66	1.03	8.33
RBDPO + 1% DGCHM	3.21	5.36	2.36	10.97	11.07	4.54	22.44	28.09	2.91	5.57	1.26	2.22
RBDPO + 3% DGCHM	3.41	5.49	2.18	10.53	10.88	4.55	23.01	27.67	2.86	5.56	1.47	2.39
RBDPO + 6% DGCHM	3.43	5.80	2.36	10.85	11.07	4.76	22.75	27.08	2.95	5.20	1.04	2.71

Table 5: Mono, di, triunsaturated and trisaturated triacylglycerol and diacylglycerol composition (%) in RBDPO added with 1, 3 or 6% DGCHM

	Mono unsaturated (SSU)	Di unsaturated (SUU)	Tri unsaturated (UUU)	Tri Saturated (SSS)	Diacylglycerol
RBDPO	40.93	37.13	6.75	6.86	4.23
RBDPO + 1% DGCHM	44.73	39.53	6.9	6.62	7.14
RBDPO + 3% DGCHM	44.11	39.81	6.73	6.96	8.00
RBDPO + 6% DGCHM	43.35	39.98	7.12	6.84	10.88

*others = OLL, MLP and SOS

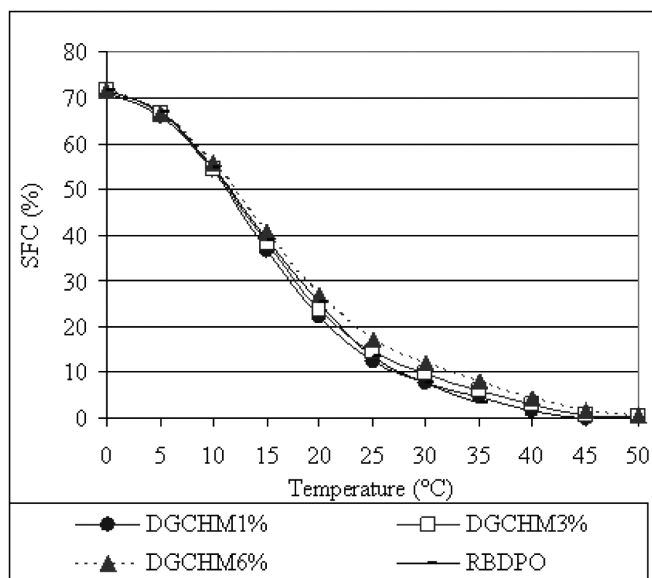
Saturated fatty acid compositions (SFAC) was higher while unsaturated fatty acid compositions (UFAC) was lower with the increased in the percentage of DGCHM addition (Table 2). The increased in SFAC accounts for the formation of the high-T peak (C) as well as the higher endset and onset temperatures of the melting and crystallization curves, respectively (Figure 1) and the decreased in IV (Table 2). This can also be attributed to the high melting and crystallization temperatures of dipalmitin in DGCHM. The increased in SFAC was more prominent especially when DGCHM addition was increased from 3 to 6 %. Therefore, the larger area of high-T peak (C) in the melting and crystallization curves of DGCHM added RBDPO could be due to the higher content of saturated fatty acid. Similar observation has also been reported in milk fat crystallization [33].

The Effect of DGCHM Addition on RBDPO: Physical Properties IV

The Effect of DGCHM Addition on RBDPO: Physical Properties IV was lower at higher percentage of DGCHM addition (Table 2). Lower IV indicated that the oil is harder. Thus in this study, increasing the percentage of DGCHM addition enhanced the formation of harder RBDPO. This is supported by higher endset and onset temperatures of the melting and crystallization curves, respectively (Figure 1). In addition, the lower IV could also be attributed to the increased in saturated fatty acids content which is due to the dipalmitin content in DGCHM. Hard fats are useful as a hardstock for margarine and shortening formulation and for the production

of specialty fats such as palm mid fractions (PMF) for use as a cocoa butter equivalent (CBE) [34].

SFC was slightly higher in the presence of DGCHM compared to RBDPO especially at 3 and 6 % DGCHM addition (Figure 3). However, the addition of 1 % DGCHM resulted in similar SFC to that of RBDPO. This indicated that the addition at 1 % level has lesser solidification effect on RBDPO compared to the 3 and 6 % addition. At 3 and 6 % addition, the presence of dipalmitin in DGCHM could have caused co-crystallization to occur and accelerated the crystallization of RBDPO TAG leading to the formation of higher amount of solid. This is inline with the earlier crystallization of RBDPO with the addition of 3 and 6 % DGCHM as shown by higher onset temperatures in the crystallization curves (Fig 1). The higher SFC in the presence of DGCHM could also be associated with the high content of dipalmitin as depicted by the high content of saturated fatty acid in DGCHM (Table 2).



DGCHM: diacylglycerol chemical method
SFC: solid fat content

Figure 3: SFC (%) of RBDPO added with 1, 3 or 6% DGCHM

From the results obtained, synthesis of dipalmitin (DP) by glycerolysis of palmitic acid in the presence of isooctane followed by neutralization and fractionation for purification is a better route to obtain DP of reasonable purity since it gives considerably high yield and requires short preparation time. The study also showed that dipalmitin promoted the crystallization of RBDPO especially at 3 and 6% addition.

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